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(11) **EP 0 818 467 A2**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
14.01.1998 Bulletin 1998/03

(51) Int. Cl.⁶: **C07K 17/02**, **C07K 14/705**,
C12N 9/12, **G01N 33/543**

(21) Application number: **97111868.2**

(22) Date of filing: **11.07.1997**

(84) Designated Contracting States:
**AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE**

(30) Priority: **12.07.1996 JP 183140/96**

(71) Applicant: **NEC CORPORATION**
Tokyo (JP)

(72) Inventors:
• **Miwa, Johji**
c/o NEC Corporation
Tokyo (JP)

• **Siddiqui, Shahid Saeed**
Kitayama-cho Toyohashi-shi Aichi (JP)

(74) Representative:
Glawe, Delfs, Moll & Partner
Patentanwälte
Postfach 26 01 62
80058 München (DE)

Remarks:

The applicant has subsequently filed a sequence
listing and declared, that it includes no new matter.

(54) **Aligned peptide array and a rational and rapid method for the detection of a binding or
interaction site of a protein by using the same**

(57) The present invention relates to an aligned peptide array comprising, as separate elements, a plurality of peptide segments obtained by dividing the amino acid sequence of a protein into sequence segments of any suitable length and any suitable overlapping frame and synthesizing peptides on the basis of said sequence segments, the amino acid sequences of said peptide segments expressing the amino acid sequence of said protein. According to the present invention, the binding or interaction site of a protein with a ligand therefor can be detected rationally, rapidly, analytically, systematically, and conveniently. It is very obvious that the concept used for the present invention can be applied to make arrays for any molecules for detection of any binding or interacting molecules.

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Description

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to aligned peptides and their immobilized preparations. It also relates to rational and rapid methods for detecting the binding or interaction site of a protein with a ligand therefor (i.e., a substance which binds to or interacts with the protein), or for the detection of such a ligand, by using the same, to methods for the modification or design of a protein or a ligand by utilizing information on a site so detected, and to immunoassay methods.

2. Description of the Related Art

Conventionally, there has been no method for rationally, rapidly, and systematically detecting the binding or interaction site of a protein with a ligand therefor.

For example, the conventional detection of a specific protein by using an antigen-antibody reaction is generally carried out on the basis of the presence of reaction with the protein as a whole, whether the antibody used is polyclonal or monoclonal. In this case, therefore, (1) the detection of the reaction does not necessarily lead to the detection of the binding site and, moreover, (2) it cannot be known how many binding sites actually exist. Moreover, in the case of detection by western blotting or detection *in situ* (e.g., in cells or tissues) by immune antibody techniques, (3) it is frequently impossible to know whether the binding protein is the specifically desired protein to be detected or not.

The present invention is based on an entirely new conception and there is no existing technique that is directly comparable to it.

As described above, because there has been no method for the direct rational and rapid detection of the binding or interaction site of a protein with a ligand therefor, the modification of a protein or ligand and the design of a new protein or ligand has depended on chance. Accordingly, such tasks have been carried out by relying on mere chance or the "intuition" of an expert.

SUMMARY OF THE INVENTION

An object of the present invention is to solve the above-described problems by providing a method for rationally, rapidly, systematically, and conveniently detecting the binding or interaction site of a protein with a ligand therefor. Another object of the present invention is to provide a rational and rapid method for detecting a ligand by using the above method. Still another object of the present invention is to provide rational and rapid methods for the modification or design of a protein or a ligand by utilizing information obtained by the above described methods, as well as by immunoassay methods.

To accomplish the above objectives, the present inventors have made various investigations and have now completed the present invention.

According to a first aspect of the present invention, there is provided an aligned peptide array comprising, as separate elements, a plurality of peptide segments obtained by dividing the amino acid sequence of a protein into sequence segments of any suitable length and of any suitable frame and synthesizing peptides on the basis of the sequence segments.

According to a second aspect of the present invention, there is provided an aligned peptide array comprising, as separate elements, a plurality of peptide segments obtained by dividing the amino acid sequence of a protein into sequence segments of any suitable length and of any suitable frame and synthesizing peptides on the basis of the sequence segments, the amino acid sequences of the peptide segments expressing the amino acid sequence of said protein.

According to a third aspect of the present invention, there is provided an immobilized aligned peptide array preparation obtained by immobilizing the aligned peptide array in accordance with the first or second aspect of the present invention.

According to a fourth aspect of the present invention, there is provided a rational and rapid method for the detection of a binding or interaction site of a protein which comprises detecting the binding or interaction site of any suitable protein or a specific protein with a ligand therefor by using the aligned peptide array in accordance with the first or second aspect of the present invention or an immobilized aligned peptide array preparation derived therefrom.

According to a fifth aspect of the present invention, there is provided a rational and rapid method for the detection of a ligand for a protein which comprises a step using the detection method in accordance with the fourth aspect of the present invention.

According to a sixth aspect of the present invention, there is provided a rational and rapid method for the modifica-

tion of a protein which comprises a step using the detection method in accordance with the fourth aspect of the present invention.

According to a seventh aspect of the present invention, there is provided a rational and rapid method for the design of a protein which comprises a step using the detection method in accordance with the fourth aspect of the present invention.

According to an eighth aspect of the present invention, there is provided a rational and rapid method for the modification of a ligand detected by the detection method in accordance with the fifth aspect of the present invention which comprises a step utilizing the information on a binding or interaction site of a protein as obtained by the detection method in accordance with the fourth aspect of the present invention.

According to a ninth aspect of the present invention, there is provided a rational and rapid method for the design of a ligand detected by the detection method in accordance with the fifth aspect of the present invention which comprises a step utilizing the information on a binding or interaction site of a protein as obtained by the detection method in accordance with the fourth aspect of the present invention.

According to a tenth aspect of the present invention, there is provided a rational and rapid immunoassay method which comprises using the aligned peptide array in accordance with the first or second aspect of the present invention or an immobilized aligned peptide array preparation derived therefrom.

According to a eleventh aspect of the present invention, there is provided an array comprising suitably selected known molecules which is used to detect binding or interacting molecules.

According to the present invention, the binding or interaction site of a protein with a ligand therefor can be detected, rationally, rapidly, systematically, and conveniently.

The detection of a ligand for a specific protein or its binding site as described in Example 2 of the present invention is not only indispensable for identification and understanding of the mode of action of the protein at a molecular level, but also very useful in the design of a protein having new properties by modifying its binding site as described in Example 3. In Example 3, for instance, a protein having the ability to combine with a phorbol ester was converted into a protein not having that ability. If this conception is extended, the reverse conversion will also be possible. Moreover, if the phorbol ester is taken as a ligand, this procedure may be applied to the detection of any desired protein and a ligand therefor.

Furthermore, if a ligand-binding or interaction site can be identified and understood at a molecular level, it will become easier to design or modify the ligand. As a result, it will become correspondingly easier to design, for example, a chemical agent or physiologically active substance that can act directly on a specific protein by recognizing it as a ligand.

Thus, the present invention also serves to create a protein or ligand having a more desirable function by modifying or designing the ligand-binding or ligand-interaction site of the protein or by modifying or designing the ligand.

Moreover, the present invention makes it possible to search for unknown ligands for any desired protein rationally, rapidly, systematically, deliberately, exhaustively, and economically.

Furthermore, the present invention can provide an excellent immunoassay method because, on the basis of an antigen-antibody reaction, the reacting (i.e., the binding or interacting) peptide segment(s) can readily be observed with the naked eye, a microscope, or other detection devices.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In the present invention, the amino acid sequence of a protein is divided into sequence segments of any suitable length and of any suitable overlapping frame, and peptides corresponding to the respective sequence segments (hereinafter referred to as "peptide segments") are synthesized. The individual peptide segments so synthesized are arranged in suitable order, for example, starting from a terminus (i.e., the amino or carboxyl terminus) of the protein. Then, they are immobilized on a suitable substance, or made into solutions and held in a suitable container. This plurality of suitably arranged peptide segments is called an aligned peptide array or a peptall or PEPTALL.

For example, an aligned peptide array can be formed by suitably arranging peptide segments so as to give the amino acid sequence of a protein as shown in the following formula (I). In this formula, the peptide units separated by "/" are independent peptide segments. Although each peptide segment consists of 10 amino acids in this example, the number of amino acids and the frame of peptide segments in a protein may be arbitrarily chosen.

MAQAENACRL//KLLRADVPVD//LLPAGCSATD//LQPAVNVKEK//IEVNGESRLV//

QKKKTLYPEW//EKCWDTAVAE//RILQIVLMFN//QPVVEATMRL//EDIISKCKSD

(SEQ ID NO:1-10)

(I)

At present, if each peptide segment consists of up to several tens of amino acids, several tens of peptide segments can be concurrently synthesized in a short period of time by use of a single peptide synthesizer. Moreover, there are an increasing number of studies demonstrating that a protein may be regarded as an aggregate consisting of several to several tens of relatively short functional peptides.

Substances suitable for the purpose of immobilizing the aligned peptide array of the present invention include membranous solid substances such as membrane filters. They also include gel-like substances in plate form, such as agar and polyacrylamide. Specific examples of the gel-like substances in plate form are agar media for the cultivation of bacteria which are placed in containers such as Petri dishes. Moreover, the aligned peptide array of the present invention may also be made by placing its peptide segments in a container which permits them to exist separately from each other. Specifically, the aligned peptide array may be made by preparing solutions containing its peptide segments and placing them in microtiter wells so that they exist separately from each other. Furthermore, the aligned peptide array of the present invention may be made in the form of a microchip or microdevice comprising an integrated circuit on which the aligned peptide array is arranged and immobilized.

According to the present invention, based on the basic conception that the amino acid sequence of a protein is divided into peptide units of any suitable length and of any suitable overlapping frame, a variety of detailed information about peptide segments corresponding to the peptide units can be acquired by carrying out various detailed experiments (including testing and designing) on an aligned peptide array comprising the regularly arranged peptide segments. In other words, this means that detailed information about any desired portion of the original protein can be obtained. Thus, the knowledge and information required for the modification of the original protein or the design of a new protein can be obtained rationally, rapidly, analytically, systematically, and easily. Moreover, a ligand for any desired protein can be detected, and knowledge and information on the ligand-binding or ligand-interaction site of the protein can be obtained rationally, rapidly, analytically, and systematically. Thus, the knowledge and information required for the modification of the ligand or the novel designing of a ligand can be obtained rationally, rapidly, analytically, and systematically.

The term "ligand" as used herein denotes any substance that binds to or interacts with proteins or any other molecules. Examples thereof include antibodies; chemical agents such as pharmaceutical drugs, agricultural chemicals and insecticides; physiologically active substances such as toxins pheromones and hormones; and biological substances such as other proteins, nucleic acids (e.g., DNA and RNA), carbohydrates, and lipids.

Obviously, the concept used for the present invention can be applied to make arrays for any molecules for detection of any binding or interacting molecules.

The present invention can also be applied to immunoassay methods. Examples thereof include enzyme immunoassay typified by ELISA, viroimmunoassay, metalloimmunoassay, fluoroimmunoassay and radioimmunoassay.

In these immunoassay methods, an antibody may first be modified with an appropriate substance (i.e., an enzyme, bacteriophage, metal, fluorescent substance or radioactive isotope) and then reacted with an aligned peptide to detect its antibody-binding or interaction site. Alternatively, an antibody may first be reacted with an aligned peptide and then modified with such a substance prior to detection. The purpose of the modification is to facilitate the observation of the site of reaction with the antibody (i.e., the binding or interaction site). That is, instead of detecting the antibody directly, the antibody is detected with detection sensitivity enhanced by the use of such an appropriate modifier.

The present invention is further illustrated by the following examples. These examples, however are not to be construed as limiting the scope of the invention.

Example 1

The present invention is explained in connection with an example in which it is applied to the detection of the binding site of a specific protein with an antibody against the protein (i.e., the antibody recognition site of the protein). In this example, it was tried to detect the binding site of tubulin, which is a protein constituting microtubules, with an antibody against it (i.e., anti-tubulin antibody).

First of all, the amino acid sequence of alpha 3-tubulin derived from a nematode was divided into 45 sequence segments each consisting of 10 amino acids (except the final sequence segment consisting of 12 amino acids), as shown

in the following formula (II).

(1) QREVISIHIG (2) QAGVQIGNAC (3) WELCYCLEHGI (4) QPDGQMPSDK
 5 (5) SLGGSDDDFS (6) TFFSETGSGR (7) HVPRAVNVDL (8) EPTVIDEIRT
 (9) GTYRSLFHPE (10) QLITGKEDAA (11) NNYARGHYTI (12) GKEEIIDLTL
 10 (13) DRIRRLADNC (14) TGLQGFLVFH (15) SFGGGTGSGF (16) TSLLNRLSV
 (17) DYGKKAKLEF (18) SIYPAPQVST (19) AVVEPYNSIL (20) TTHTTLEHSD
 (21) CSFNVDNEAI (22) YDICRRNLDI (23) ERPSYTNLNR (24) LIGQIVSSIT
 15 (25) ASLRFDGALN (26) VDLTEFQTNL (27) VPYPRIHFPL (28) ATFSPVISAE
 (29) KAYHEQLSVA (30) EITNNCFEPH (31) NQNVKCDPRH (32) RGDVVPKDVN
 20 (33) RGDVVPKDVN (34) AAIATIKTKR (35) SIQFVDWCPT (36) GFKYVGINYQ
 (37) PPTVVPGGDL (38) AKVPRAVCML (39) SNTTAIAEAW (40) ARLDHKFDLM
 25 (41) YAKRAVHWY (42) VGEGMEEGEF (43) SEAREDLAAL (44) EDKYEEVGVD
 (45) SMEDNGEEGDEY (SEQ ID NO: 11-55) (II)
 30

Next, peptide segments were synthesized according to the amino acid sequences of the respective sequence segments. These peptide segments were arranged and immobilized on a membrane filter to prepare an aligned peptide array membrane.

This aligned peptide array membrane was first reacted with anti-acetylated tubulin antibody, and then with a secondary antibody comprising mouse anti-rabbit IgG tagged with horseradish peroxidase. Thereafter, the reaction spot was identified by staining the aligned peptide array membrane by means of a color development reaction.

40 In this example, color development was observed in the peptide segments corresponding to the fourth and fifth sequence segments of formula (II).

It can be concluded from the above results that the binding site of alpha 3-tubulin with the anti-acetylated tubulin antibody used in this example lies in the amino acid sequence QPDGQMPSDKSLGGSDDDFS.

45 Example 2

First of all, an amino acid sequence corresponding to a portion of the regulatory region of protein kinase C (PKC) was divided as shown in the following formula (III), and the corresponding peptide segments were synthesized. Then, an aligned peptide array membrane was prepared in the same manner as given in Example 1.

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(1) VHEIRGHQFVATFFR (2) QPHFCSLCSDFMWGL (3) NKQGYQCQLCSAAVH
 (4) KKCHEKVIMQCPGSA (5) KNTKETMALKERFKV (6) DIPHRFKTYNFKSPT
 (7) FCDHCGSMLYGLFKQ (8) GLRCEVCNVACHHKC (9) ERLMSNLCGVNQKQL
 (SEQ ID NO:56-64) (III)

The aligned peptide array membrane was reacted with a phorbol ester labeled with tritium (^3H) and then brought into contact with a photographic film. Thus, the reaction spot was identified by autoradiography.

In this example, exposure to radiation was observed at the position of the peptide segment corresponding to the third sequence segment of formula (III). This sequence segment corresponds to a "zinc finger-like" sequence that has conventionally been presumed to be a phorbol ester-binding site.

Example 3

In the third peptide segment detected in Example 2, one amino acid was replaced as shown in the following formula (IV). Excepting this modification, detection was carried out in the same manner as in Example 2.

NKQGYQCQLCSAAVH \rightarrow NKQEYQCQLCSAAVH (SEQ ID NO:65) (IV)

As a result, no exposure to radiation was observed at the position of the third peptide segment, in which one amino acid had been replaced. This fact can be interpreted to mean that, in consequence of the replacement of G (glycine) in the third peptide segment by E (glutamate), the phorbol ester acting as a ligand lost its ability to bind to this region.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANTS:

10

- (A) NAME: NEC CORPORATION
- (B) STREET: 7-1, SHIBA 5-CHOME, MINATO-KU,
- (C) CITY: TOKYO
- (D) STATE:
- (E) COUNTRY: JAPAN
- (F) POSTAL CODE :

15

- (ii) TITLE OF INVENTION: Aligned peptide array and a rational and rapid method for the detection of a binding or interaction site of a protein by using the same

20

- (iii) NUMBER OF SEQUENCES: 65

(v) COMPUTER READABLE FORM:

25

- (A) MEDIUM TYPE: Floppy
- (B) COMPUTER: PC
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: ASCII Text

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(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: EP97111868.2
- (B) FILING DATE: 11/07/97

(2) INFORMATION FOR SEQ ID NO:1

35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Met Ala Gln Ala Glu Asn Ala Cys Arg Leu
1 5 10

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(2) INFORMATION FOR SEQ ID NO:2

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:2:

Lys Leu Leu Arg Ala Asp Val Pro Val Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:3:

Leu Leu Pro Ala Gly Cys Ser Ala Thr Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:4:

Leu Gln Pro Ala Val Asn Val Lys Glu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:5

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:5:

Ile Glu Val Asn Gly Glu Ser Arg Leu Val
1 5 10

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(2) INFORMATION FOR SEQ ID NO:6

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:6:

Gln Lys Lys Lys Thr Leu Tyr Pro Glu Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO:7

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:7:

Glu Lys Cys Trp Asp Thr Ala Val Ala Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:8:

Arg Ile Leu Gln Ile Val Leu Met Phe Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:9:

Gln Pro Val Val Glu Ala Thr Met Arg Leu
1 5 10

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(2) INFORMATION FOR SEQ ID NO:10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:10:

Glu Asp Ile Ile Ser Lys Cys Lys Ser Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:11

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:11:

Gln Arg Glu Val Ile Ser Ile His Ile Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:12

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:12:

Gln Ala Gly Val Gln Ile Gly Asn Ala Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:13

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:13:

Trp Glu Leu Tyr Cys Leu Glu His Gly Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:14

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:14:

Gln Pro Asp Gly Gln Met Pro Ser Asp Lys
 1 5 10

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:15:

Ser Leu Gly Gly Ser Asp Asp Ser Phe Ser
 1 5 10

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:16:

Thr Phe Phe Ser Glu Thr Gly Ser Gly Arg
 1 5 10

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(2) INFORMATION FOR SEQ ID NO:17

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:17:

His Val Pro Arg Ala Val Asn Val Asp Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:18

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:18:

Glu Pro Thr Val Ile Asp Glu Ile Arg Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:19:

Gly Thr Tyr Arg Ser Leu Phe His Pro Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:20:

Gln Leu Ile Thr Gly Lys Glu Asp Ala Ala
1 5 10

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(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:21:

Asn Asn Tyr Ala Arg Gly His Tyr Thr Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:22

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:22:

Gly Lys Glu Glu Ile Ile Asp Leu Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:23

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:23:

Asp Arg Ile Arg Arg Leu Ala Asp Asn Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:24

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:24:

Thr Gly Leu Gln Gly Phe Leu Val Phe His
1 5 10

(2) INFORMATION FOR SEQ ID NO:25

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:25:

Ser Phe Gly Gly Gly Thr Gly Ser Gly Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO:26

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:26:

Thr Ser Leu Leu Asn Glu Arg Leu Ser Val
 1 5 10

(2) INFORMATION FOR SEQ ID NO:27

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:27:

Asp Tyr Gly Lys Lys Ala Lys Leu Glu Phe
 1 5 10

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(2) INFORMATION FOR SEQ ID NO:28

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:28:

Ser Ile Tyr Pro Ala Pro Gln Val Ser Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:29

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:29:

Ala Val Val Glu Pro Tyr Asn Ser Ile Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:30:

Thr Thr His Thr Thr Leu Glu His Ser Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:31

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:31:

Cys Ser Phe Asn Val Asp Asn Glu Ala Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:32

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:32:

Tyr Asp Ile Cys Arg Arg Asn Leu Asp Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:33

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:33:

Glu Arg Pro Ser Tyr Thr Asn Leu Asn Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:34

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:34:

Leu Ile Gly Gln Ile Val Ser Ser Ile Thr
1 5 10

EP 0 818 467 A2

(2) INFORMATION FOR SEQ ID NO:35

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:35:

Ala Ser Leu Arg Phe Asp Gly Ala Leu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:36

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:36:

Val Asp Leu Thr Glu Phe Gln Thr Asn Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:37

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:37:

Val Pro Tyr Pro Arg Ile His Phe Pro Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:38

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:38:

Ala Thr Phe Ser Pro Val Ile Ser Ala Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:39

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:39:

Lys Ala Tyr His Glu Gln Leu Ser Val Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:40:

Glu Ile Thr Asn Asn Cys Phe Glu Pro His
1 5 10

(2) INFORMATION FOR SEQ ID NO:41

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:41:

Asn Gln Asn Val Lys Cys Asp Pro Arg His
1 5 10

EP 0 818 467 A2

(2) INFORMATION FOR SEQ ID NO:42

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:42:

Arg Gly Asp Val Val Pro Lys Asp Val Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:43

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:43:

Arg Gly Asp Val Val Pro Lys Asp Val Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:44

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:44:

Ala Ala Ile Ala Thr Ile Lys Thr Lys Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:45

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:45:

Ser Ile Gln Phe Val Asp Trp Cys Pro Thr
1 5 10

EP 0 818 467 A2

(2) INFORMATION FOR SEQ ID NO:46

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:46:

Gly Phe Lys Tyr Val Gly Ile Asn Tyr Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:47

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:47:

Pro Pro Thr Val Val Pro Gly Gly Asp Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:48

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:48:

Ala Lys Val Pro Arg Ala Val Cys Met Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:49

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:49:

Ser Asn Thr Thr Ala Ile Ala Glu Ala Trp
1 5 10

(2) INFORMATION FOR SEQ ID NO:50

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:50:

Ala Arg Leu Asp His Lys Phe Asp Leu Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:51

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:51:

Tyr Ala Lys Arg Ala Phe Val His Trp Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:52

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:52:

Val Gly Glu Gly Met Glu Glu Gly Glu Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:53

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:53:

Ser Glu Ala Arg Glu Asp Leu Ala Ala Leu
 1 5 10

(2) INFORMATION FOR SEQ ID NO:54

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:54:

Glu Lys Asp Tyr Glu Glu Val Gly Val Asp
 1 5 10

(2) INFORMATION FOR SEQ ID NO:55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:55:

Ser Met Glu Asp Asn Gly Glu Glu Gly Asp Glu Tyr
 1 5 10

(2) INFORMATION FOR SEQ ID NO:56

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:56:

15 Val His Glu Ile Arg Gly His Gln Phe Val Ala Thr Phe Phe Arg
 1 5 10 15

20 (2) INFORMATION FOR SEQ ID NO:57

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:57:

30 Gln Pro His Phe Cys Ser Leu Cys Ser Asp Phe Met Trp Gly Leu
 1 5 10 15

35 (2) INFORMATION FOR SEQ ID NO:58

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:58:

45 Asn Lys Gln Gly Tyr Gln Cys Gln Leu Cys Ser Ala Ala Val His
 1 5 10 15

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EP 0 818 467 A2

(2) INFORMATION FOR SEQ ID NO:59

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:59:

Lys Lys Cys His Glu Lys Val Ile Met Gln Cys Pro Gly Ser Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:60

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:60:

Lys Asn Thr Lys Glu Thr Met Ala Leu Lys Glu Arg Phe Lys Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:61

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:61:

Asp Ile Pro His Arg Phe Lys Thr Tyr Asn Phe Lys Ser Pro Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:62

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:62:

Phe Cys Asp His Cys Gly Ser Met Leu Tyr Gly Leu Phe Lys Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:63

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:63:

15 Gly Leu Arg Cys Glu Val Cys Asn Val Ala Cys His His Lys Cys
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:64

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:64:

30 Glu Arg Leu Met Ser Asn Leu Cys Gly Val Asn Gln Lys Gln Leu
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:65

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION:SEQ ID NO:65:

45 Asn Lys Gln Glu Tyr Gln Cys Gln Leu Cys Ser Ala Ala Val His
 1 5 10 15

50 Claims

1. An aligned peptide array comprising, as separate elements, a plurality of peptide segments obtained by dividing the amino acid sequence of a protein into sequence segments of any suitable length and of any suitable frame and synthesizing peptides on the basis of said sequence segments.
- 55 2. An aligned peptide array comprising, as separate elements, a plurality of peptide segments obtained by dividing the amino acid sequence of a protein into sequence segments of any suitable length and of any suitable frame and synthesizing peptides on the basis of said sequence segments, the amino acid sequences of said peptide segments

expressing the amino acid sequence of said protein.

3. An immobilized aligned peptide array preparation obtained by immobilizing the aligned peptide array of claim 1 or 2.

5 4. An aligned peptide array membrane comprising a membranous solid substance on which the aligned peptide array of claim 1 or 2 is immobilized.

5. An immobilized aligned peptide array preparation comprising a gel-like substance on which the aligned peptide array of claim 1 or 2 is immobilized.

10 6. An aligned peptide array plate comprising a gel-like substance in the form of a plate on which the aligned peptide array of claim 1 or 2 is immobilized.

15 7. An immobilized aligned peptide array preparation comprising a container in which the peptide segments constituting the aligned peptide array of claim 1 or 2 are placed in such a way that they exist separately from each other.

8. An immobilized aligned peptide array preparation, comprising a series of microtiter wells in which solutions containing the peptide segments constituting the aligned peptide array of claim 1 or 2 are placed in such a way that they exist separately from each other.

20 9. A microchip comprising an integrated circuit on which the aligned peptide array of claim 1 or 2 is immobilized.

10. A microdevice comprising an integrated circuit on which the aligned peptide array of claim 1 or 2 is immobilized.

25 11. A method for the detection of a binding or interaction site of a protein which comprises detecting the binding or interaction site of any suitable protein or a specific protein with a ligand therefor by using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.

30 12. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is an antibody.

13. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is a chemical agent.

35 14. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is a physiologically active substance.

15. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is another protein.

40 16. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is a nucleic acid.

45 17. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is a carbohydrate.

18. A method for the detection of a binding or interaction site of a protein as claimed in claim 11 wherein the ligand is a lipid.

50 19. A method for the detection of a ligand for a protein which comprises a step using the detection method of claim 11.

20. A method for the modification of a protein which comprises a step using the detection method of claim 11.

21. A method for the design of a protein which comprises a step using the detection method of claim 11.

55 22. A method for the modification of a ligand detected by the detection method of claim 19 which comprises a step utilizing the information on a binding or interaction site of a protein as obtained by the detection method of claim 11.

23. A method for the design of a ligand detected by the detection method of claim 19 which comprises a step utilizing the information on a binding or interaction site of a protein as obtained by the detection method of claim 11.
- 5 24. An immunoassay method which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
25. An enzyme immunoassay method, represented by ELISA, which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
- 10 26. A viroimmunoassay method which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
27. A metalloimmunoassay method which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
- 15 28. A fluoroimmunoassay method which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
29. A radioimmunoassay method which comprises using the aligned peptide array of claim 1 or 2 or an immobilized aligned peptide array preparation derived therefrom.
- 20 30. An array comprising suitably selected known molecules which is used to detect binding or interacting molecules.
31. An immobilized array preparation obtained by immobilizing the array of claim 30.
- 25 32. An array membrane comprising a membranous solid substance on which the array of claim 30 is immobilized.
33. An immobilized array preparation comprising a gel-like substance on which the array of claim 30 is immobilized.
- 30 34. An array plate comprising a gel-like substance in the form of a plate on which the array of claim 30 is immobilized.
- 35 35. An immobilized array preparation comprising a container in which the molecules constituting the array of claim 30 are placed in such a way that they exist separately from each other.
36. An immobilized array preparation, comprising a series of microtiter wells in which solutions containing the molecules constituting the array of claim 30 are placed in such a way that they exist separately from each other.
37. A microchip comprising an integrated circuit on which the array of claim 30 is immobilized.
- 40 38. A microdevice comprising an integrated circuit on which the array of claim 30 is immobilized.